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(54) Title: LABELLING OF NUCLEIC ACID ANALOGUE-PEPTIDE CHIMERAE

(57) Abstract

A nucleic acid analogue of the PNA type is provided with a kemptide motif and radio-labelled by phosphorylation at a service residue in said motif to provide a radio-labelled nucleic acid analogue having a specific activity in excess of 1×10^5 cpm/μg ^{32}P.

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Labelling of Nucleic Acid Analogue-Peptide Chimerae

The present invention relates to the production of labelled nucleic acid analogues and their use in analytical procedures.

Nucleic acid analogues having important new utilities in assay procedures and in the field of diagnostics have been described in WO 92/20703. These nucleic acid analogues had a number of new properties making them of special importance in the field of diagnostics as well as in the field of antisense therapeutics.

They typically feature a polyamide backbone bearing a sequence of ligands which are nucleic acid bases. The analogues described there have the property of hybridising with great specificity and stability to natural nucleic acids of complementary sequence.

In order to aid the detection and the manipulation of such nucleic acid analogues in diagnostic or other assay procedures and the like operations, it is desirable to provide the nucleic acid analogues with detectable labels.

It has been proposed to radio-label such nucleic acid analogues. Other labelling techniques have been proposed also. We have now developed certain chimeric structures in which a nucleic acid analogue as previously described is linked to a peptide motif (i.e. a series of peptide bonded amino acids) selected such that the chimera undergoes a convenient labelling reaction.

Accordingly, the present invention provides in a first aspect, a method for labelling a nucleic acid analogue comprising providing a nucleic acid analogue with a peptide motif capable of functioning as a substrate for an enzyme in a labelling reaction and carrying out a said labelling reaction comprising reacting the peptide motif of the nucleic acid analogue under the influence of an enzyme with a source of said label. Preferably, said label is a radio-label, preferably radio-labelled ATP.

The radio-active atom in the radio-label is preferably ^{32}P at the γ position.

Alternatively, however the label may be any other detectable moiety which is attachable to a peptide in an enzyme mediated reaction, e.g. a biotin label.

The enzyme is preferably a protein kinase.

The peptide motif is preferably the kemptide motif, i.e. H-leu-Arg-Arg-Ala-Ser-Leu-Gly-. It is known that this motif when present in a protein or peptide will undergo a labelling reaction in which it acts as a substrate for the action of protein kinase A and is phosphorylated at the serine residue. Other phosphorylatable motifs may also be used. These include abbreviated kemptide motifs, e.g. the first five amino acid residues of the kemptide motif H-Arg-Ala-Ser-Leu-Gly-.

Preferably therefore, the labelling reaction is a phosphorylation at a serine residue of said peptide motif.

The nucleic acid analogue is preferably one comprising a polymeric strand which includes a sequence of ligands bound to a backbone made up of linked backbone moieties, which analogue is capable of hybridisation to a nucleic acid of complementary sequence, and further comprises said peptide motif.

Said nucleic acid analogue backbone is preferably a polyamide, polythioamide, polysulphinamide or polysulphonamide backbone.

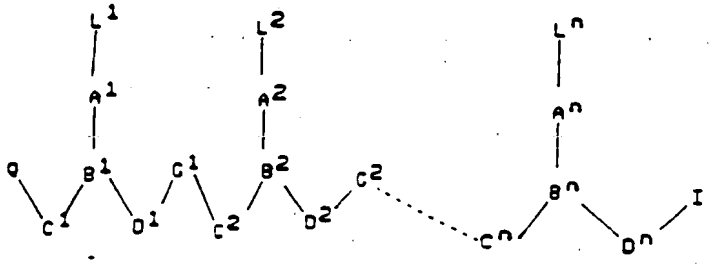
Preferably, said linkded backbone moieties are peptide bonded amino acid moieties and preferably said peptide motif is present at the N-terminus or the C-terminus.

The nucleic acid analogue is preferably capable of hybridising to a nucleic acid of complementary sequence to form a hybrid which is more stable against denaturation by heat than a hybrid between the conventional deoxyribonucleotide corresponding in sequence to said analogue and said nucleic acid.

Preferably, said nucleic acid analogue is a peptide nucleic acid in which said backbone is a polyamide backbone, each said ligand being bonded directly or indirectly to an aza nitrogen atom in said backbone, and said ligand bearing nitrogen atoms mainly being separated from one another in said backbone by from 4 to 8 intervening atoms.

Preferably also, the nucleic acid analogue is capable of hybridising to a double stranded nucleic acid in which one strand has a sequence complementary to said analogue, in such a way as to displace the other strand from said one strand.

Preferably, the nucleic acid analogue has the general formula 1:



Formula 1

wherein:

n is at least 2,

each of L^1-L^n is independently selected from the group consisting of hydrogen, hydroxy, (C_1-C_4)alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and said peptide motif, but normally at least one L will be a nucleobase binding group such as a naturally occurring nucleobase and preferably at least 90 % of the groups L will be such nucleobase binding groups;

each of C^1-C^n is $(CR^6R^7)y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined below, and R^5 is hydrogen, (C_1-C_6)alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

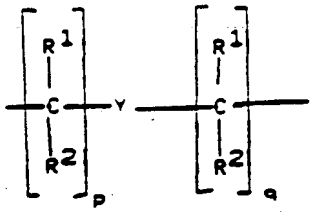
each of D^1-D^n is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above;

each of y and z is zero or an integer from 1 to 10, the sum y + z being from 2 to 10 (preferably more than 2, and preferably such that each of x and y is 1 or 2);

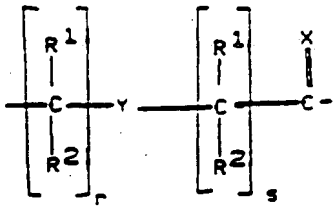
each of G^1-G^{n-1} is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined below;

each of A^1-A^n and B^1-B^n are selected such that:

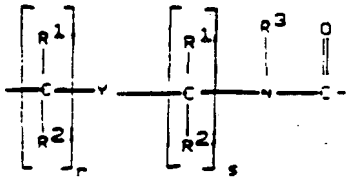
(a) A is a group of formula (IIa), (IIb), (IIc) or (IId), and B is N or R^3N^+; or
(b) A is a group of formula (IId) and B is CH;



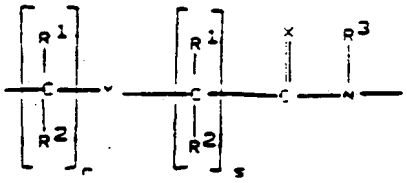
Formula IIa



Formula IIb



Formula IIc



Formula IId

wherein:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;
Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p + q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r + s being not more than 10;

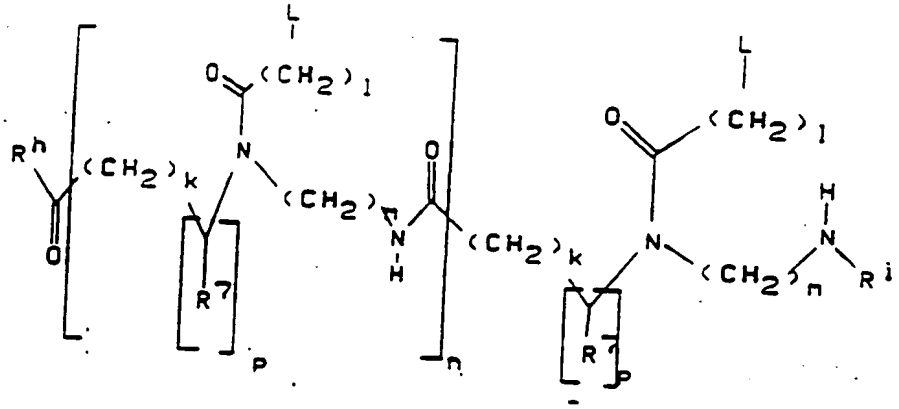
each R^1 und R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen; and

each R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;

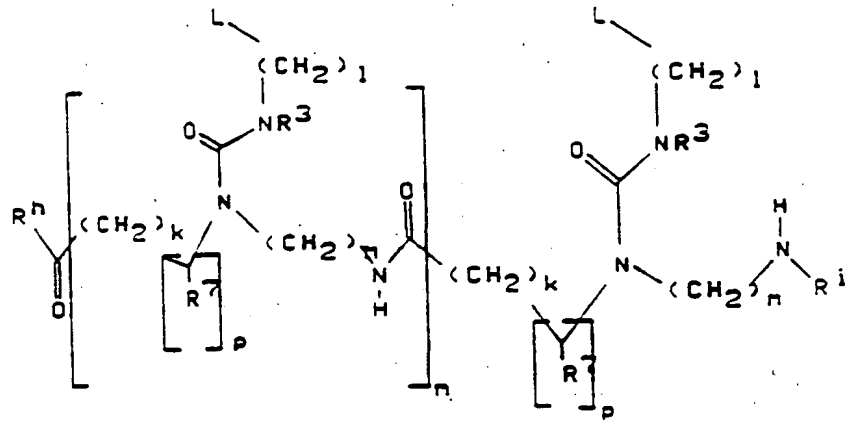
Q is $-CO_2H$, -CONR'R", $-SO_3H$ or $-SO_2$NR'R" or an activated derivative of $-CO_2H$ or $-SO_3H$; and

I is -NR'R'", where R' and R" are indenpendently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers, and -R'" is an R" group or said peptide motif, at least one L group or -R'" being said peptide motif.

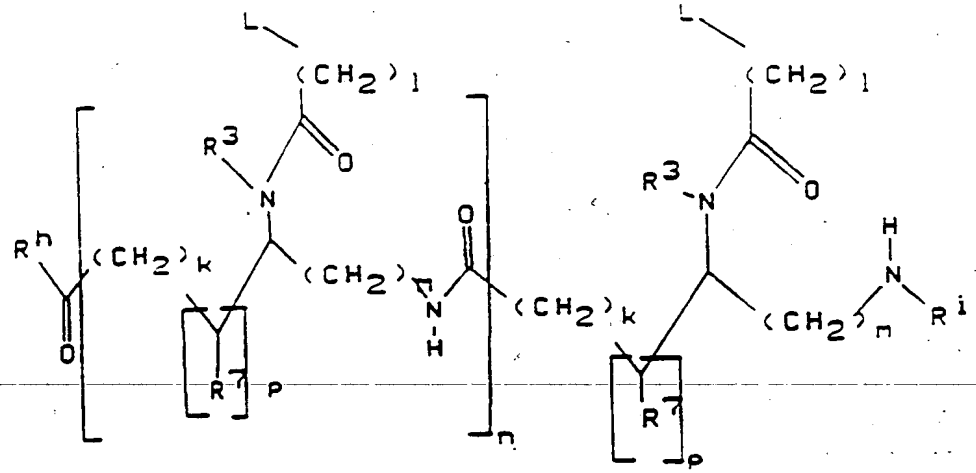
More preferably, said nucleic acid analogue comprises a compound of the general formula III, IV or V:



Formula III



Formula IV



Formula V

wherein:

each L is independently selected from the group consisting of hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, and non-naturally occurring nucleobases;

each R^7 is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids;

n is an integer greater than 1,

each k, l, and m is, independently, zero or an integer from 1 to 5;

each p is zero or 1;

R^h is OH, NH_2 or -NHLysNH$_2$; and
R^i is said peptide motif.

Preferably the label is a ^{32}P label.

Preferably, the label is contained is a phosphate group attached to a serine residue which preferably forms part of a peptide motif.

The peptide motif preferably includes the kemptide motif.

In a third aspect, the invention provides a nucleic acid analogue comprising a polymeric strand which includes a sequence of ligands bound to a backbone made up of linked backbone moieties, which analogue is capable of hybridisation to a nucleic acid of complementary sequence, further comprising a peptide motif capable of acting as a substrate for an enzyme in a labelling reaction.

Preferably, said peptide motif is reactable with radio-labelled ATP to phosphorylate said peptide motif in the presence of a protein kinase and preferably therefore the peptide motif is the kemptide motif.

Preferably, the nucleic acid analogue is as described above in connection with the first aspect of the invention.

In a fourth aspect the invention provides an analytical method comprising hybridising a radio-labelled nucleic acid analogue produced by a method as described with reference to the first aspect of the invention or being in accordance with the second aspect of the invention, to a nucleic acid and detecting the presence of the hybrids so produced by the radio-label.

Preferably, the nucleic acid to be detected is bound to a support and is probed using said labelled nucleic acid analogue.

The invention will be further described and illustrated by the following description of preferred features of the invention and by the examples in which reference is made to the accompanying drawings in which:

Figure 1 is an autoradiograph produced in example 3 below.

Figure 2 is an autoradiograph produced in example 4 below.

PNA's having peptide extensions of desired amino acid sequences can conveniently be produced by the Boc or Fmoc solid phase techniques well understood in the art once a starting PNA sequence has been built-up on a suitably solid support by using the Boc solid phase synthesis described in WO 92/20703. Alternatively, the peptide extension may be synthesised first before the PNA sequence is started.

The amino acid sequence of the peptide motif may be the kemptide motif for radio-labelling. For biotin labelling it may be a sequence as described in "Biotechnology", Vol 11, Oct. 1993, pp 1138-1142 by P.J. Schatz. Suitably the sequence is:

Leu-x-Leu-Zle-Phe-Glu-Ala-Gln-Lys-Zle-Glu-Trp-Arg which is biotinylated by the E. Coli biotin holoenzyme synthetase at the Lysine residue of biotin so supplied as biotinyl-5'-adenylate or biotin ad ATP.

The biotin can be used as a label detectable by avidin or streptavidin or may itself carry a radio-label such as ^{3}H.

Figure 3 shows a structure of PNA (peptide nucleic acid) molecules compared to normal DNA.

Figure 4 shows the sequence and nucleic acid hybridization characteristics of some of the PNA's used herein.

Figure 5 shows an HPLC analysis of a time study of the enzymatic labelling of the molecules according to the invention.

Example 1

Preparation of a PNA-kemptide chimera

The solid phase PNA synthesis described in WO 92/20703 was used to build up the sequence:

Boc-NH$(CH_2)_5$CONH-TG(Z)T.A(Z)C(Z)G(Z).TC(Z)A(Z).C(Z)A(Z)A(Z).C(Z)TA(Z)-CONH-resin

The N terminal Boc group was removed by treatment with TFA and used as a starting point for a standard boc type solid phase peptide synthesis of the kemptide motif via the linker 6-amino-hexanoic acid to produce the chimera:

Boc-Leu-Arg(Tos)-Arg(Tos)-Ala-Ser-(Bzl)-Leu-Gly-NH$(CH_2)_5$-CONH-TG(Z)T.A(Z)C(Z)G(Z).TC(Z)A(Z).C(Z)A(Z) A(Z).C(Z)TA(Z)-CONH-resin

The protection groups were removed and the product cleaved from the resin by the Low-High TFMSA procedure. The raw product was purified by preparative HPLC (reversed phase C_{18} eluting with a gradient of A:0.1% TFA in water (MilliQ) and B:0.1% TFA, 10% water, 90% acetonitrile). The purified chimeric PNA-kemptide was characterized by analytical HPLC and FAB-MS.

Example 2

The kemptide motif (Leu-Arg-Arg-Ala-Ser-Leu-Gly) functions as a substrate for protein kinase A covalently attached to a PNA

The PNA-kemptide chimera of the formula:

H-Leu-Arg-Arg-Ala-Ser-Leu-Gly-TGTACGTCACAACTA-NH_2 was labelled with ^{32}P in a reaction mixture containing:

PNA-kemptide, 10 µM	5 µl
10 x Protein Kinase A buffer	5 µl
γ ^{32}P ATP (>5000 Ci/mmol; 50 µCi/µl)	10 µl
Protein Kinase A (Boehringer; 5 mU/µl)	0.2 µl
H_2O	30 µl

The reaction was incubated for 30 minutes at 30°C and then for 10 minutes at 65°C before being centrifuged for 30 seconds at 15000 g. The supernatant was transferred to a new Eppendorff tube. Water was added to 1 ml and the labelled PNA-kemptide was separated from unincorporated γ ^{32}P ATP by anion exchange chromatography using a DEAE Sephadex A-50 anion exchange column.

The specific activity of the PNA-kemptide was estimated at 1 x 10^8 cpm/µg PNA-kemptide.

Example 3

The hybridization properties of PNA are retained in a PNA-kemptide chimera

The abililty of unlabelled/^{32}P labelled PNA62-kemptide to hybridise to its complementary unlabelled/^{32}P labelled oligonucleotide in solution was analysed by gelshift in a 20% non-denaturing polyacrylamide gel. Hybridisation stringency was controlled by the addition of formamide, which suppresses the T_m of a PNA 62/DNA duplex at about ½°C/1% formamide. Hybridisations were carried out in a 20 µl reaction volume containing 10 mM Tris-Cl, pH 8.0, 1 mM EDTA, and PNA, DNA and formamide as indicated in the figure legend. Hybridisation mixtures were incubated for 15 min. at 37°C. At the end of the incubation period 4 µl loading buffer (50% glycerol, 5 x TBE buffer and 0.25% (w/v) bromphenolblue) was added to the reaction mix subsequent to which the samples were loaded on to a 20% non-denaturating polyacrylamide gel/1 x TBE and electrophoresed at 400 V for 1 hour. Finally the gel was subjected to autoradiography. Figure 1 shows the results of the hybridisation analyses. Lane 1: Labelled oligonucleotide alone (migration control). Lanes 2-4: Control PNA62 (PNA62 without the kemptide addition) incubated with labelled, complementary oligonculeotide in the presence of 0% (2), 30% (3) and 60% (4) formamide. Lanes 5-7: Unlabelled PNA62-kemptide incubated with labelled, complementary oligonucleotide in the presence of 0% (5), 30% (6) and 60% (7) formamide. Lanes 8-10: ^{32}P labelled PNA62-kemptide incubated with unlabelled, complementary

oligonucleotide in the presence of 0% (5), 30% (6) and 60% (7) formamide. In conclusion these results show that:

1. The addition of the kemptide extension to PNA62 does not significantly alter its ability to hybridise with a complementary oligonucleotide.
2. Unlabelled and ^{32}P labelled PNA62-kemptide exhibits similar hybridisation properties.
3. A PNA carrying the kemptide motif is a substrate for phosphorylation by protein kinase A.

Example 4

^{32}P labelled PNA62-kemptide can be used as a probe to detect complementary DNA fragments bound to a filter membrane

The labelled PNA62-kemptide was used as a probe to detect complementary sequences in a DNA fragment immobilised on a membrane (Southern hybridisation). DNA-framgents containing a sequence complementary to the PNA62-kemptide and DNA fragments containing a sequence with a single mismatch to the PNA62-kemptide were generated by PCR amplification of the appropriate plasmids. The DNA fragments were separated by gel-electrophoresis in a 1% TAE agarose gel and transferred to a Hybond N+ membrane by standard alkali blotting procedures. The filter was prehybridised at 50°C in a rotary oven in 5 ml hybridisation solution (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) containing 4 ng unlabelled PNA T10-kemptide as a blocking agent for unspecific attachment of the probe to the filter. After 1 hour of prehybridisation, 5 µl of 32P labelled PNA62-kemptide was added to the prehybridisation solution and incubation was continued at 50°C for 16 hours. The filter was washed twice in 10 mM Tris-HCl pH 7½, 1 mM EDTA for 30 min. at 50°C, air dried, and exposed to autoradiography for 10 hours. As shown in figure 1, the PNA62-kemptide probe hybridises efficiently to the fully complementary DNA fragments on the filter (lane 4). Furthermore, it hybridises to some extent to the DNA fragment that carries the C to T point mutation (lane 2). This is expected because the PNA62 G/T DNA mismatched duplex in solution has a T_m of 61°C which is above the stringency level imposed on the hybridisation. In contrast to the G to T mutation, the C to G and C to A mutations have a major effect on the stability of the resulting PNA/DNA duplex in solution (PNA62 G/A DNA: Tm = 51°C and PNA62 G/G DNA: Tm = 53°C). Consistent with these T_m data, the PNA-kemptide probe does not hybridise to the corresponding DNA fragments

on the filter (lane 1 and 3, respectively). In conclusion, the results shows that a labelled PNA-kemptide can be used as a probe in a filter hybridisation assay and that such probes are able to discriminate effectively between the fully complementary and single base mismatched target nucleic acids. Similar results were obtained using other membranes (Gene Screen + Immobilon S) and other blocking reagents (1 % Casein and 1 %Triton X-100).

Example 5

Functional analysis of the peptide segment of the chimera

The chimera (20 pmol) and control DNA $(Ado)_3$-PNA) (20 pmol) were incubated separately in a reaction volume (50 µl) containg ^{32}P γ-ATP[1] (Amersham) (100 pmol > 5000 Ci/mmol), 50 mM MES (pH 6.9), 10 mM $MgCl_2$ 0.5 mM EDTA, 1 mM DTT, 1 mg/mL BSA and 5 mU PKA1 (Boehringer Mannheim). After 30 min at 30°C the PNAs were separated from unincorporated γ-ATP by ion exchange chromatography using diethylaminoethyl (DEAE) Sephadex A-50[1] (Sigma) and the purified PNAs were counted the control PNA. The sample containing the chimera had a specific activity of 2.4×10^6 cpm/pmol PNA. When using ^{32}P γ-ATP at a specific acitivity of 5000 Ci/mmol the calculated maximum possible specific activity of the chimera would be 6×10^6 dpm/pmol.

The enzymatic phosphorylation of the chimera was studied in a time course experiment. 1 mU PKA was used to phosphorylate 1 nmol chimera. Samples were taken at different time points during the reaction and analyzed on HPCL. Within 60 s ca. 20 % of the chimera had been phosphorylated (Fig. 5) and more than 50 % had been phosphorylated after 120 s. the reaction was completed within 300 s. The identity of the phosphorylated product was confirmed by mass spectroscopy (ESI; calculated/found: 5371.1/5370.9).

Claims

1. A method for labelling a nucleic acid analogue comprising providing a nucleic acid analogue with a peptide motif capable of functioning as a substrate for an enzyme in a labelling reaction and carrying out a said labelling reaction comprising reacting the peptide motif of the nucleic acid analogue under the influence of an enzyme with a source of said label.

2. A method as claimed in claim 1, wherein said label is a radio-label.

3. A method as claimed in claim 1, wherein the source of said label is radio-labelled ATP.

4. A method as claimed in claim 3, wherein said enzyme is a protein kinase.

5. A method as claimed in any preceding claim, wherein the peptide motif is the kemptide motif.

6. A method as claimed in any one of claims 1 to 5, wherein the labelling reaction is phosphorylation at a serine residue of said peptide motif.

7. A method as claimed in any preceding claim, wherein the nucleic acid analogue comprises a polymeric strand which includes a sequence of ligands bound to a backbone made up of linked backbone moieties, which analogue is capable of hybridisation to a nucleic acid of complementary sequence, and further comprises said peptide motif.

8. A method as claimed in claim 7, wherein said nucleic acid analogue backbone is a polyamide, polythioamide, polysulphinamide or polysulphonamide backbone.

9. A method as claimed in claim 8, wherein said linked backbone moieties are peptide bonded amino acid moieties.

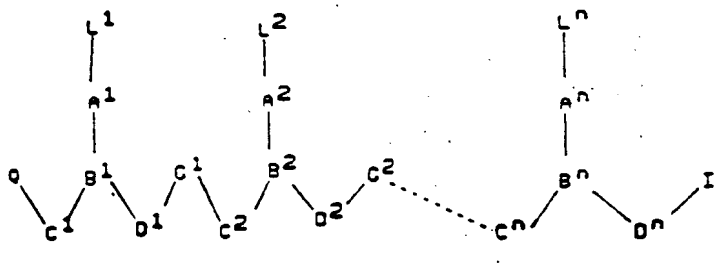
10. A method as claimed in claim 9, wherein said peptide motif is present at the N-terminus or is present at the C-terminus.

11. A method as claimed in any preceding claim, wherein the nucleic acid analogue is capable of hybridising to a nucleic acid of complementary sequence to form a hybrid which is more stable against denaturation by heat than a hybrid between the conventional deoxyribo-nucleotide corresponding in sequence to said analogue and said nucleic acid.

12. A method as claimed in any preceding claim, wherein said nucleic acid analogue is a peptide nucleic acid in which said backbone is a polyamide backbone, each said ligand being bonded directly or indirectly to an aza nitrogen atom in said backbone, and said ligand bearing nitrogen atoms mainly being separated from one another in said backbone by from 4 to 8 intervening atoms.

13. A method as claimed in any preceding claim, wherein the nucleic acid analogue is capable of hybridising to a double stranded nucleic acid in which one strand has a sequence complementary to said analogue, in such a way as to displace the other strand from said one strand.

14. A method as claimed in any preceding claim, wherein the nucleic acid analogue has the general formula 1:



Formula 1

wherein:

n is at least 2,

each of L^1-L^n is independently selected from the group consisting of hydrogen, hydroxy, (C_1-C_4)alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and said peptide motif;

each of C^1-C^n is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, $(C_2$-$C_6)$alkyl, aryl, aralkyl, heteroaryl, hydroxy, $(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined below, and R^5 is hydrogen, $(C_1$-$C_6)$alkyl, hydroxy, alkoxy, or alkylthio-substituted (C_1 to C_6)alkyl or R^6 and R^7 taken together complete an alicyclid or heterocyclic system;

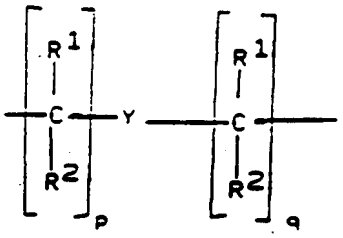
each of D^1-D^n is $(CR^6R^7)_z$ where R^66 and R^7 are as defined above;

each of y and z is zero or an integer from 1 to 10, the sum y + z being from 2 to 10;

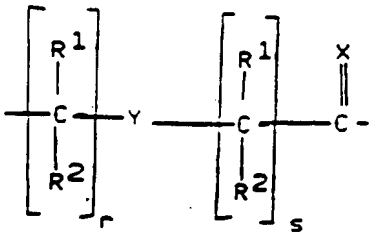
each of G^1-G^n is $-NR^3CO-$, $-NR^3C^S-$, $-NR^3SO-$ or $-NR^3\ SO_2-$, in either orientation, where R^3 is as defined below;

each of A^1-A^n and B^1-B^n are selected such that:

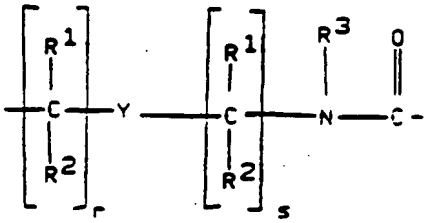
(a) A is a group of formula (IIa), (IIb), IIc) or (IId), and B is N or R^3N+; or
(b) A is a group of formula (IId) and B is CH;



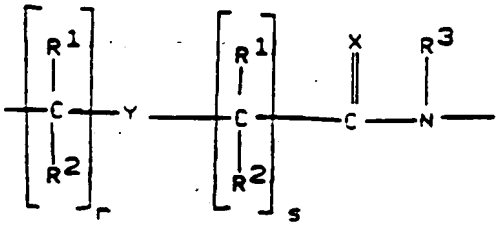
Formula IIa



Formula IIb



Formula IIc



Formula IId

wherein:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;
Y is a single bond, O, S or NR^4;

each of p an q is zero or an integer from 1 to 5, the sum p + q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r + s being not more than 10;

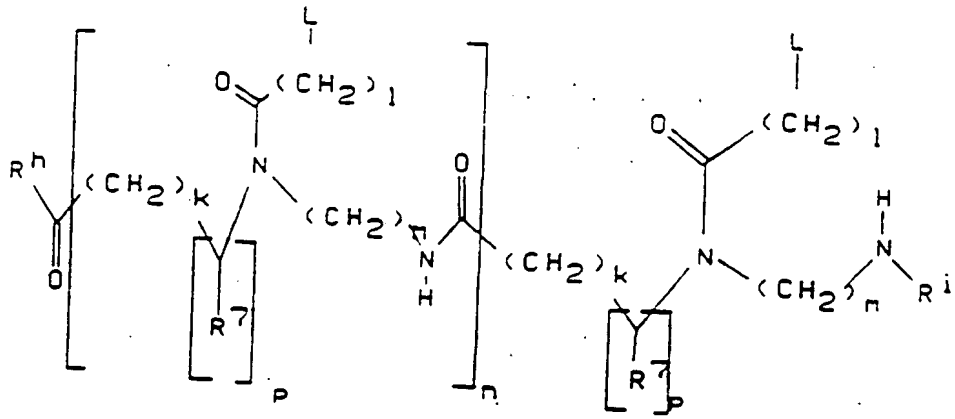
each of R^1 and R^2 is indenpendently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen; and

each R^3 and R^4 is indenpendently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;

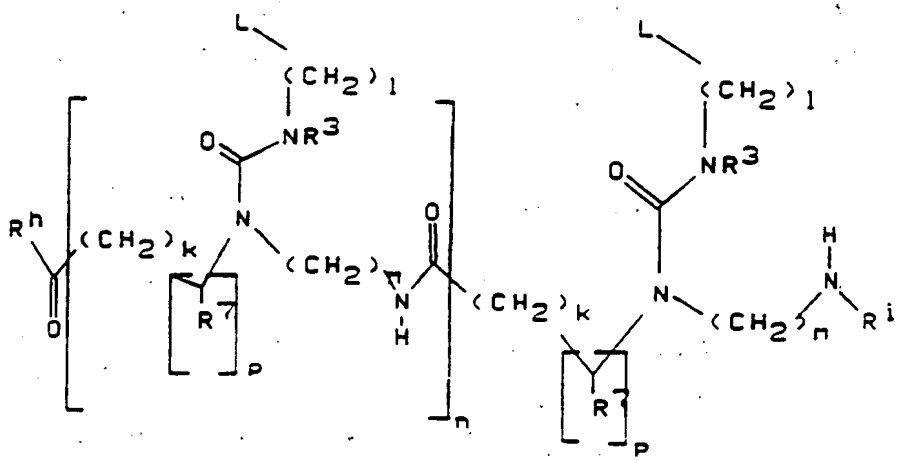
Q is $-CO_2H$, -CONR'R", $-SO_3H$ or $-SO_2NR'R"$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

I is -NR'R'", where R' and R" are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleo-sides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribo-nucleotides and
oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers, and -R'" is an R" group or said peptide motif, at least L or -R'" being said peptide motif.

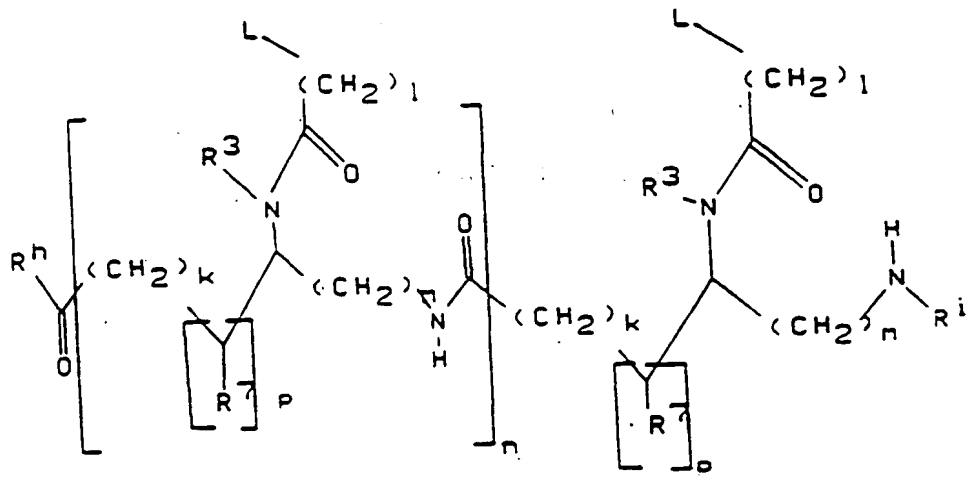
15. A method as claimed in claim 14, wherein said nucleic acid analogue comprises a compound of the general formula III, IV or V:



Formula III



Formula IV



Formula V

wherein:

each L is indenpendently selected from the group consisting of hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, and non-naturally occurring nucleobases;

each R^7 is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids;

n is an integer greater than 1,

each k, l, and m is, independently, zero or an integer from 1 to 5;

each p is zero or 1;

R^h is OH, NH_2 or -NHLysNH$_2$; and

R^i is said peptide motif.

16. A radio-labelled nucleic acid analogue having a specific activity in excess of 1×10^5 cpm/µg.

17. A labelled nucleic acid analogue as claimed in claim 16, wherein the label is a ^{32}P label.

18. A labelled nucleic acid analogue as claimed in claim 17, wherein the label is contained in a phosphate group attached to a serine residue.

19. A labelled nucleic acid analogue as claimed in claim 18, wherein said serine residue forms part of a peptide motif.

20. A labelled nucleic acid analogue as claimed in claim 19, wherein said peptide motif includes the kemptide motif.

21. A nucleic acid analogue comprising a polymeric strand which includes a sequence of ligands bound to a backbone made up of linked backbone moieties, which analogue is capable of hybridisation to a nucleic acid of complementary sequence, further comprising a peptide motif capable of acting as a substrate for an enzyme in a labelling reaction.

22. A nucleic acid analogue as claimed in claim 21, wherein said peptide motif is reactable with radio-labelled ATP to phosphorylate said peptide motif in the presence of a protein kinase.

23. A nucleic acid analogue as claimed in claim 22, wherein the peptide motif is the kemptide motif.

24. A nucleic acid analogue as claimed in any one of claims 16 to 21, wherein the backbone is a polyamide, polythioamide, polysulphinamide or polysulphonamide backbone.

25. A nucleic acid analogue as claimed in claim 24, wherein said linked backbone moieties are peptide bonded amino acid moieties.

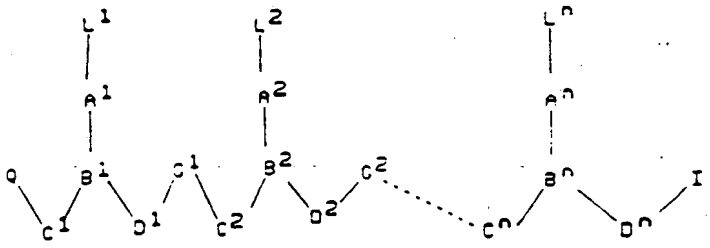
26. A nucleic acid analogue as claimed in claim 24 or claim 25, wherein said peptide motif is present at the N-terminus or is present at the C-terminus.

27. A nucleic acid analogue as claimed in any one of claims 16 to 26, wherein the nucleic acid analogue is capable of hybridising to a nucleic acid of complementary sequence to form a hybrid which is more stable against denaturation by heat than a hybrid between the conventional deoxyribo-nucleotide corresponding in sequence to said analogue and said nucleic acid.

28. A nucleic acid analogue as claimed in any one of claims 16 to 27, wherein the nucleic acid analogue is a peptide nucleic acid in which said backbone is a polyamide backbone, each said ligand being bonded directly or indirectly to an aza nitrogen atom in said backbone, and said ligand bearing nitrogen atoms mainly being separated from one another in said backbone by from 4 to 8 intervening atoms.

29. A nucleic acid analogue as claimed in any one of claims 16 to 28, wherein the nucleic acid analogue is capable of hybridising to a double stranded nucleic acid in which one strand has a sequence complementary to said analogue, in such a way as to displace the other strand from said one strand.

30. A nucleic acid analogue as claimed in any one of claims 16 to 29, wherein the nucleic acid analogue has the general formula 1:



Formula 1

wherein:

n is at least 2,

each of L^1-L^n is independently selected from the group consisting of hydrogen, hydroxy, (C_1-C_4)alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and peptide motifs;

each of C^1-C^n is $(CR^6R^7)y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined below, and R^5 is hydrogen, (C_1-C_6)alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

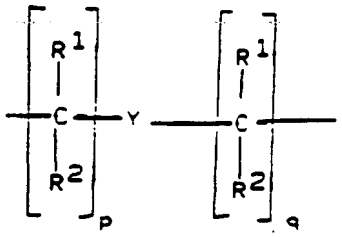
each of D^1-D^n is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above;

each of y and z is zero or an integer from 1 to 10, the sum y + z being from 2 to 10;

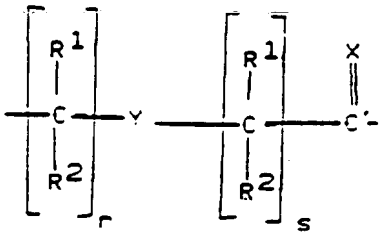
each of G^1-G^n is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined below;

each of A^1-A^n and B^1-B^n are selected such that:

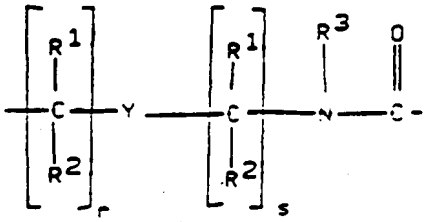
(a) A is a group of formula (IIa), (IIb), (IIc) or (IId), and B is N or R^3N^+; or
(b) A is a group of formula (IId) and B is CH;



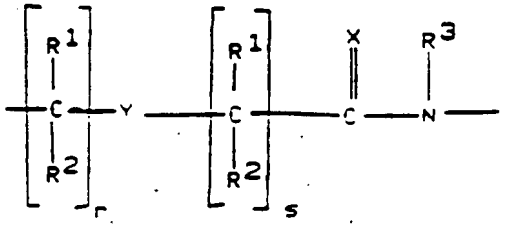
Formula IIa



Formula IIb



Formula IIc



Formula IId

wherein:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;
Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p + q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r + s being not more than 10;

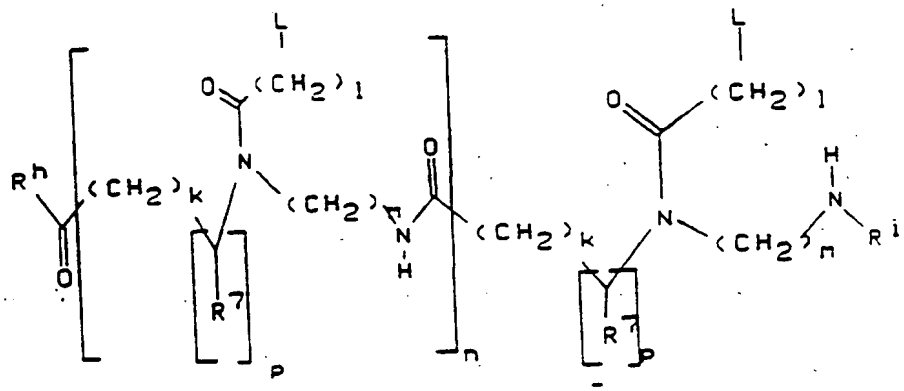
each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen; and

each 3 and R^4 is independently selected from the group consisting of hydrogen (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;

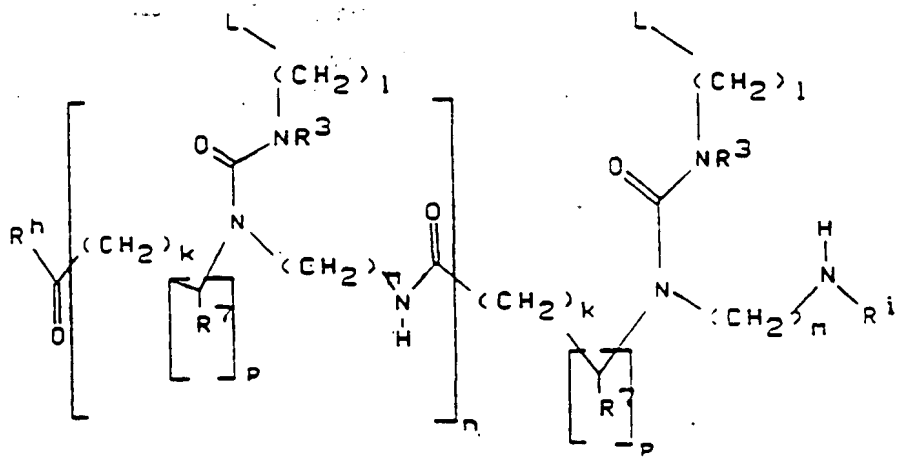
Q is $-CO_2H$, -CONR'R", $-SO_3H$ or $-SO_2NR'R"$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

I is -NR'R'", where R' and R" are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleo-sides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribo-nucleotides and
oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers, and -R'" is an -R" group or the peptide motif, at least one L or the group-R'" being said peptide motif.

31. A nucleic acid analogue as claimed in claim 11, wherein said nucleic acid analogue comprises a compound of the general formula III, IV or V:



Formula III



Formula IV

wherein:

each L is independently selected from the group consisting of hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, and non-naturally occurring nucleobases;

each R^7 is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids;

n is an integer greater than 1,

each k, l, and m is, independently, zero or an integer from 1 to 5;

each p is zero or 1;

R^h is OH, NH_2 or -NHLysNH$_2$; and
R^i is a chelating moiety.

32. A nucleic acid analogue incorporating a peptide motif, substantially as hereinbefore described in example 1.

33. An analytical method comprising hybridising a radio-labelled nucleic acid analogue produced by a method as claimed in any one of claims 1 to 15, or as claimed in any one of claims 16 to 20, to a nucleic acid and detecting the presence of the hybrids so produced by the radio-label.

34. A method as claimed in claim 33, wherein the nucleic acid to be detected in bound to a support and is probed using said labelled nucleic acid analogue.

35. A method of radio-labelling a nucleic acid analogue or a radio-labelled nucleic acid analogue substantially as hereinbefore described in example 2.

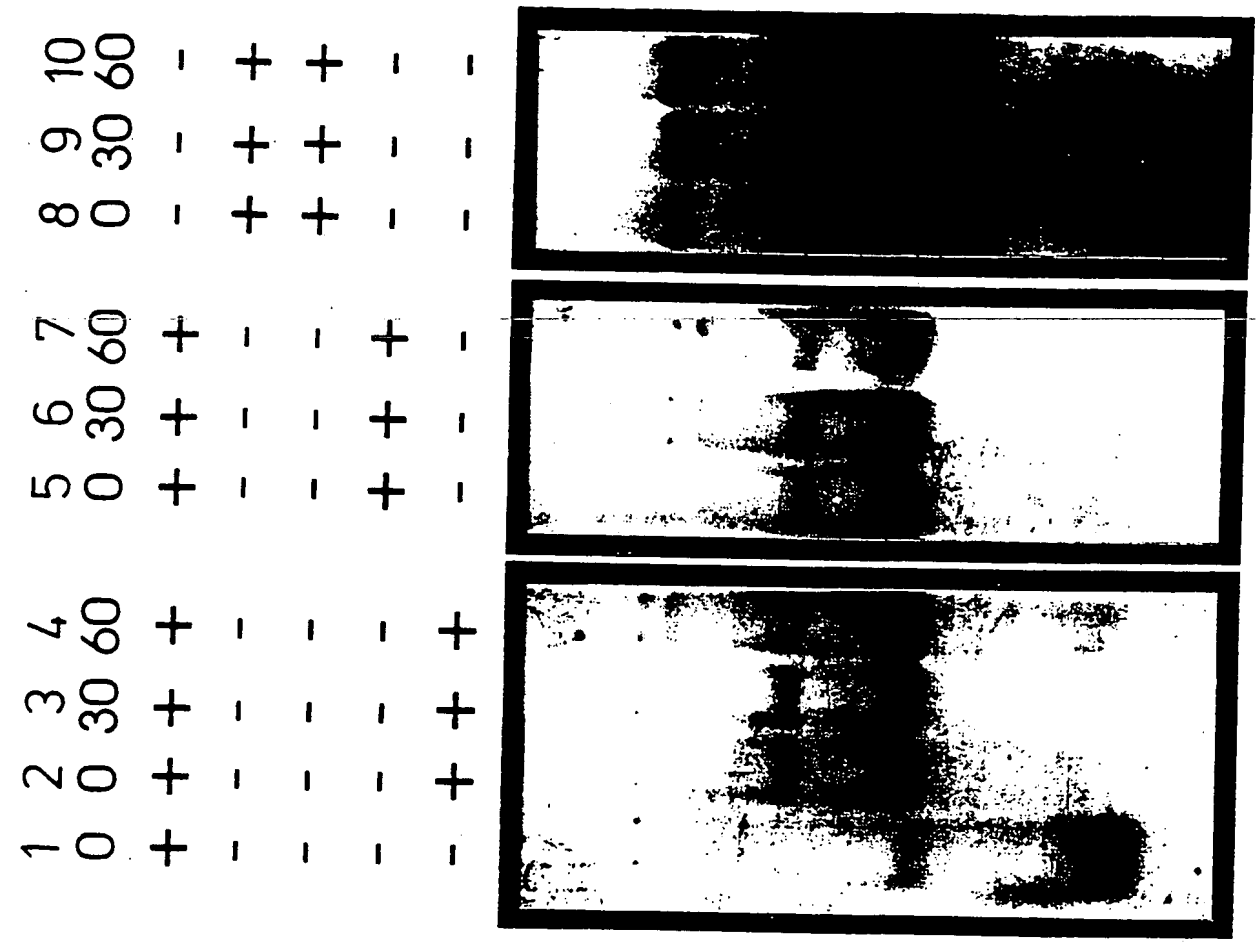
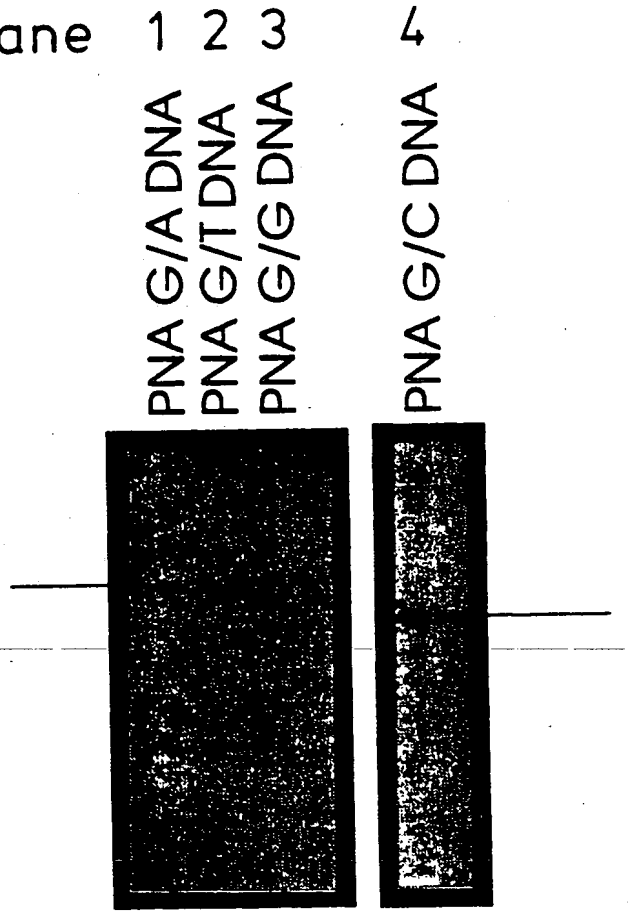

Lane
1 2 3 4 5 6 7 8 9 10
Formamid in %
0 0 30 60 0 30 60 0 30 60
32P Oligonucleotide
+ + + + + + + - - -
Oligonucleotide
- - - - - - - + + +
32P PNA62-kemptide
- - - - + + + + + +
PNA62-kemptide
- - - - + + + - - -
PNA62
- + + + - - - - - -

FIG. 1.

FIG. 2.



A
T
G
C

DNA----ACA TGC AGT GTT GAT-----5´
PNA: H-TGT ACG TCA CAA CTA-NH_2

FIG. 3

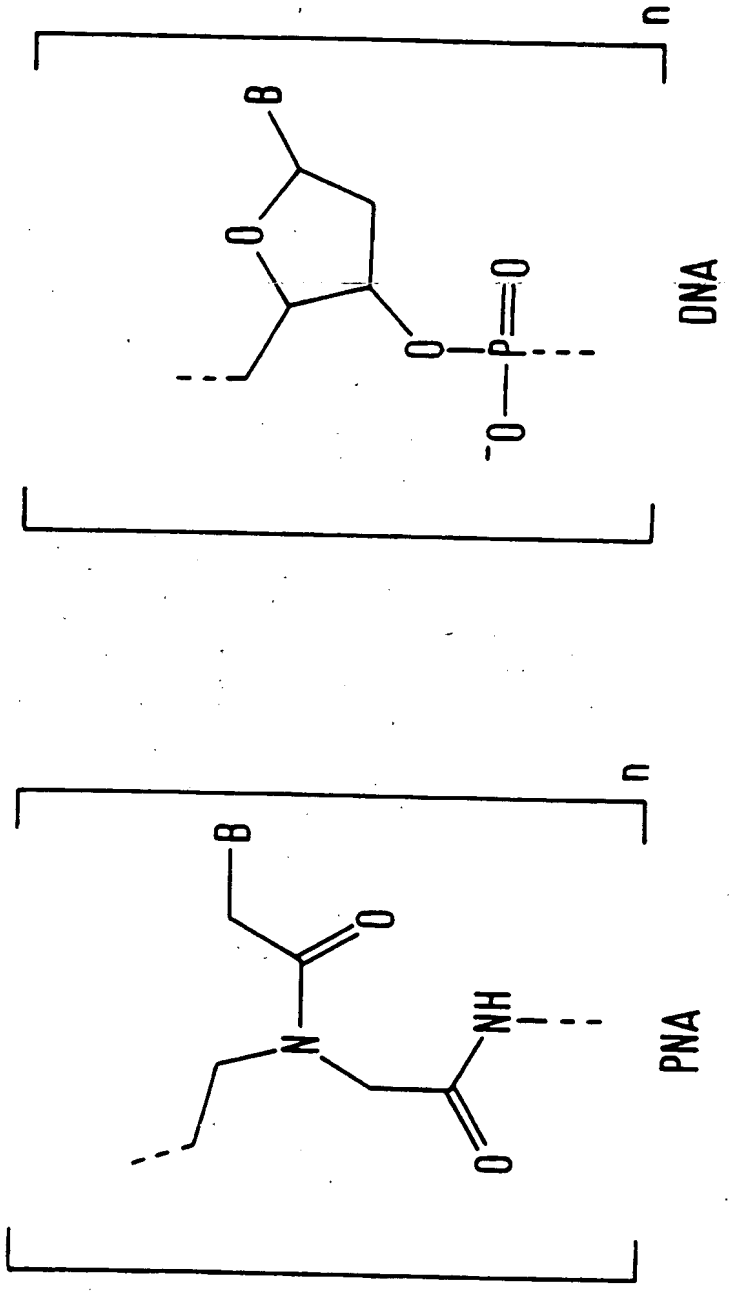
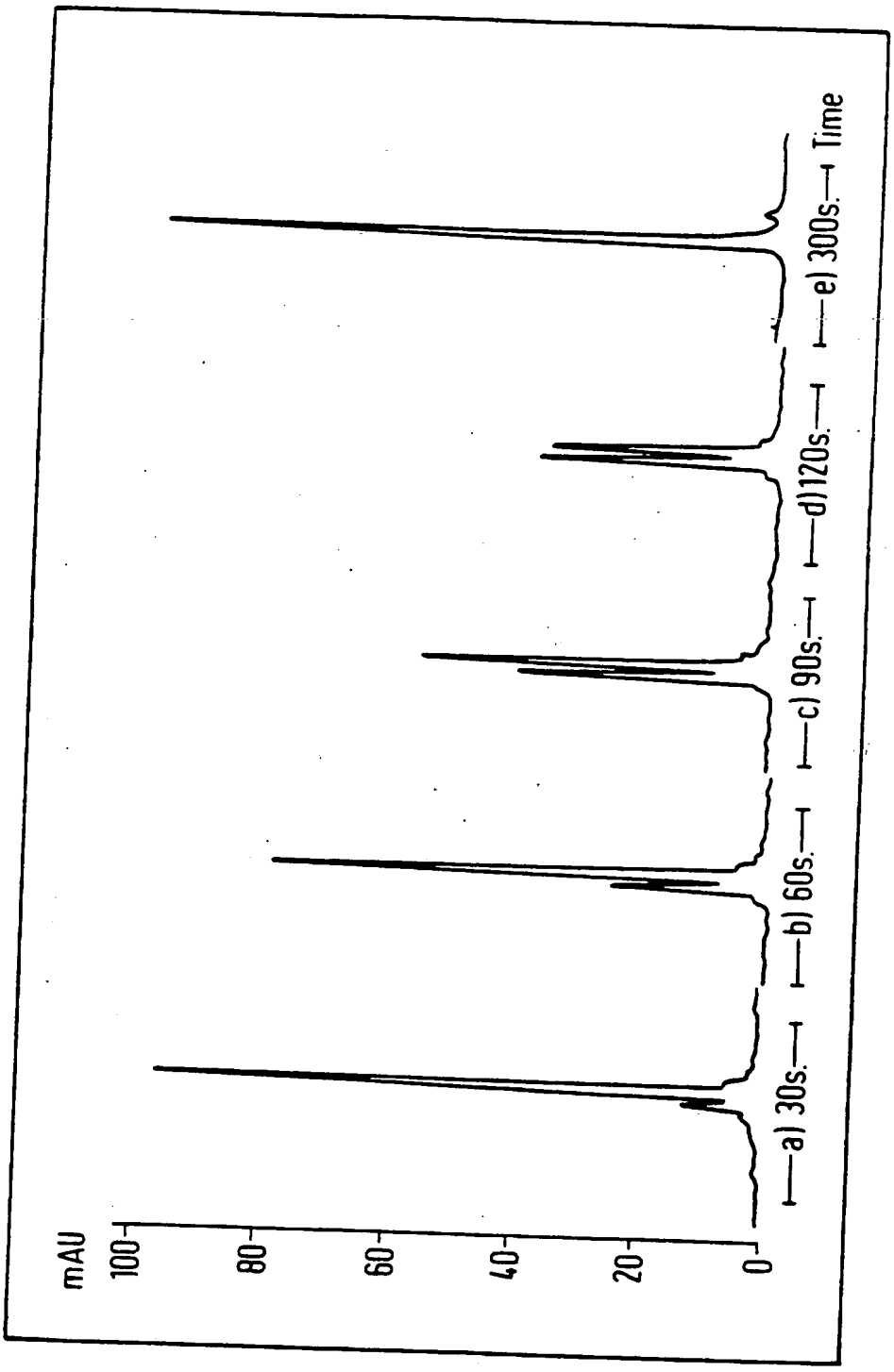


FIG. 4

Row	PNA/DNA COMPLEX	Mismatch	X=none	X=ado_3	Un-labelled X=ado_3-kemptide	Labelled X=ado_3-kemptide
1	5' - CTAGAGGATCTAGTTGTGA**C**GTACAGGATCTTTTTCATAG - 3' PNA:H_2N-ATCAACACT**G**CATGT - X	none	67.4°C	68.8°C	70.0°C	68.8°C
2	5' - CTAGAGGATCTAGTTGTGA**A**GTACAGGATCTTTTTCATAG - 3' PNA:H_2N-ATCAACACT**G**CATGT - X	G_{PNA}/A_{DNA}	51.2°C	53.1°C	55.1°C	53.9°C
3	5' - CTAGAGGATCTAGTTGTGA**T**GTACAGGATCTTTTTCATAG - 3' PNA:H_2N-ATCAACACT**G**CATGT - X	G_{PNA}/T_{PNA}	58.9°C	60.7°C	62.3°C	61.2°C
4	5' - CTAGAGGATCTAGTTGTGA**G**GTACAGGATCTTTTTCATAG - 3' PNA:H_2N-ATCAACACT**G**CATGT - X	G_{PNA}/G_{DNA}	53.1°C	53.8°C	55.9°C	54.6°C

FIG. 5



A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/533 C07K14/00 C12Q1/68 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H C12Q C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,92 20793 (BUCHARDT, EGHOLM, NIELSEN AND BERG) 26 November 1992 cited in the application see claims 1,2,6 see examples 68,69	1-35
Y	US,A,4 923 802 (GALLIS) 8 May 1990 see abstract see column 2, line 44 - line 53 see column 6, line 37 - line 57 see column 8, line 11 - line 32	1-35

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 February 1995	21. 03. 95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Scott, J

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/EP 94/03972

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9220793	26-11-92	AU-B-	654724	17-11-94
		AU-A-	1994592	30-12-92
		CA-A-	2086327	11-11-92
		EP-A-	0542971	26-05-93
		JP-T-	6500574	20-01-94
US-A-4923802	08-05-90	NONE		